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Editorial

HBV protein as a double-barrel shot-gun targets epigenetic landscape in liver cancer ☆

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Hepatitis B virus (HBV) infection is a major risk factor for developing hepatocellular carcinoma (HCC), although other environmental and lifestyle factors, including hepatitis C virus (HCV) infection, heavy alcohol consumption, chronic exposure to aflatoxins and tobacco smoking are considered to be the other associated risk factors. HBV infection, which is involved in 53% of HCC cases, affects more than 350 million people and is the most deleterious type of viral hepatitis [1]. However, although a causal relationship between HBV infection and development of HCC has been well-established, the molecular mechanisms by which HBV triggers hepatocarcinogenesis are still unclear [2–4]. Besides integration of the viral genome in the host cell chromosomes and potential activation of cellular surveillance mechanisms, accumulating evidence indicates that HBV-infected hepatocytes often exhibit altered epigenetic status [5–8]. Hence, it has been proposed that the viral genome and/or viral proteins can interfere with normal epigenetic mechanisms whose disruption may promote development of HCC [9,10]. In agreement with this hypothesis, some evidence suggests that HBV encoded 17 kDa X-protein (HBx), which is capable of transactivation and transrepression of both viral and cellular genes through its direct interaction with several different nuclear transcription factors, may modulate transcriptional activity of the target genes through epigenetic modifications [7,8,11–13]. HBx was found to upregulate expression of the DNA-methyltransferase (DNMT) genes (supporting a transcriptional transactivation property of HBx), and thus corresponding down-regulation of several important cellular genes was attributed to the DNMT-mediated methylation of the target genes [11,12]. Transcription transactivation property of HBx is supported by the fact that HBx interacts with CBP/P300 complex possessing histone acetyltransferase activity [14]. A recent study, perhaps surprisingly, revealed a histone deacetylase (HDAC) as a direct HBx-interacting partner [13], thus raising the questions of whether HBx associates with other members of the cellular epigenetic machinery and what governs these interactions.

In their current study, Zheng et al. [15] in this issue of the Journal use an elegant biochemical approach to detect interaction of HBx with cellular epigenetic machinery proteins, unraveling the mechanisms involved in HBx-mediated epigenetic modifications and gene regulation. Using microarray analysis they compared gene-expression differences between HBx-overexpressing and control hepatic cells. Among several genes whose expression was found to be affected by ectopic HBx-expression, the authors broadly concentrated on MT1F and IL4R genes (which were found to be down-regulated), and IGFBP3 and CDH6 genes among the genes showing up-regulation. Surprisingly, expressions of these targeted genes also corresponded with the methylation status of their respective promoters, indicating that HBx may affect the methylation status of regulatory regions of these genes. Possible HBx-mediated up-regulation of expression of DNMT-genes and thus resultant down-

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regulation of certain cellular genes due to promoter DNA hypermethylation could explain the hypermethylation-associated repression of the MT1F and IL4R gene expression. However, hypomethylation mediated up-regulation of IGFBP6 and CDH6 genes was still intriguing. The authors thus argue that the observed increase in DNMT activity alone cannot account for the unique and CpG-methylation-based alterations in the transcriptional activities (up- as well as down-regulation) of the specific target genes, in HBx transfected cells. They thus hypothesized that there must be some other cellular mechanism that triggers cellular chromatin remodeling and subsequent regionally aberrant DNA methylation. In order to check this hypothesis, authors took advantage of immunoprecipitation and ChIP techniques. One would expect that HBx might interact with either histone acetyltransferases (which by recruitment on the target gene regulatory sequence, may facilitate histone acetylation and up-regulation of candidate genes) or with some DNA-demethylase (which may help to up-regulate candidate gene via the observed promoter DNA hypomethylation). Indeed, previous studies reported that HBx functionally interacts with CBP/p300 (histone acetyltransferase) in regulation of CREB-mediated transcription [14]. On the contrary, one might expect to find it recruiting a DNA-methyltransferase and/or histone deacetylase on the promoter region of the HBx-suppressed candidate genes [16]. Hypermethylation of cellular genes in the presence of HBx, due to DNMT1 and DNMT3A recruitment on target promoter regions is known [11,12]. However, in the present work Zheng et al. focus mainly on HBx interaction with DNMT's and HDAC1. Contrary to previous findings they show that ectopic expression of HBx in Huh7 and HepG2 cells was unable to influence expression of DNMT1, DNMT3A or DNMT3B. Moreover, they successfully demonstrate that HBx interacts directly with DNMT1, DNMT3A and HDAC1. The DNMT3A interaction was proved to be physiologically relevant as it could also be found in HCC tissue samples.

Next, the authors addressed the importance of interaction between HBx and these epigenetic players. By employing ChIP and re-ChIP techniques they demonstrate that ectopic HBx recruits DNMT3A on promoters of the repressed genes (MT1F and IL4R) and that epigenetic modification of these promoters (i.e. hypermethylation) takes place specifically by DNMT3A recruitment (Fig. 1B). The present finding is also in agreement with the previous observation, showing that HBx specifically represses insulin-like growth factor-3 expression through DNMT3A-mediated *de novo* methylation and by inhibiting SP1 binding via recruiting methyl-CpG-binding protein 2 (MCBP2) to the newly methylated SP1 binding element [12]. Hence, in the light of the present work it can be speculated that HBx-mediated repression of the IGF-3 gene may also require recruitment of DNMT3A-HBx complex on the IGF-3 promoter region (Fig. 1B).

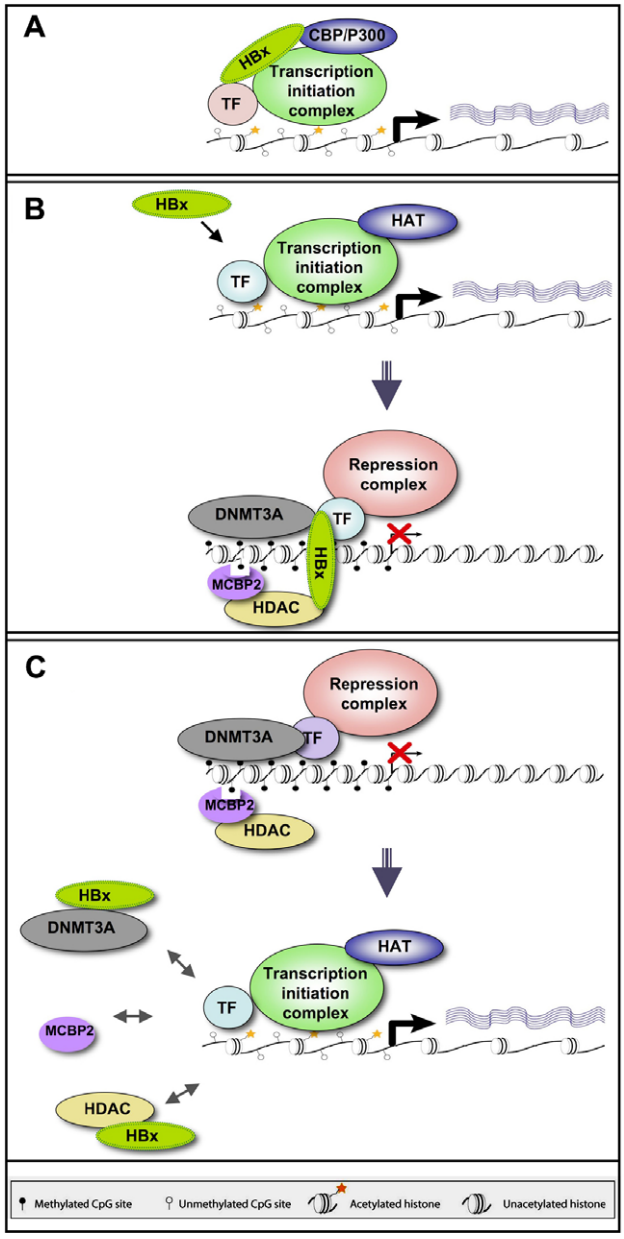

Fig. 1. Hypothetical models depicting possible molecular mechanisms by which HBx deregulate epigenetic states and expression of host genes. (A) Current understanding of HBx interactions with host cellular gene-regulatory machinery in unscheduled activation of cellular genes. HBx by interacting with DNA-bound transcription factors may help recruitment of histone acetyltransferases and thus exhibit its transactivation function e.g. IGF3 gene [14]. (B and C) The model based on the findings presented by Zheng et al. Binding of HBx to specific transcription factor(s) may lead to subsequent recruitment of DNMT3A or HDAC1, resulting in hypermethylation and heterochromatinization of the promoter region and gene silencing e.g. MT1F and IL4R genes (B). HBx may hinder with binding of potential repressor(s) on target gene-promoter and thus allowing hypomethylation, possible histone acetylation (active chromatin conformation) and expression of the target gene e.g. IGFBP3 and CDH6 genes (C). TF, transcription factor; HAT, histone acetyltransferase; MCBP, methyl-cytosine binding protein.

In human cells, DNA methylation is catalyzed by three different DNMTs. DNMT1 functions as a maintenance enzyme for the premethylation patterns, whereas, DNMT3A and DNMT3B establish genomic imprinting during embryogenesis and methylate repetitive sequences, respectively [16]. It is interesting to note that disruption of DNMT3A and DNMT3B results in neoplastic transformation and tumor progression; thus the present finding by Zheng et al. emphasizes the importance of HBV-mediated DNMT deregulation in HCC etiology, which may help the design of novel epigenetics-based therapies. Moreover, as DNA methylation is considered as a primary requirement during gene silencing, it is possible that as an initial step HBx-DNMT complexes are recruited to repress a target gene via DNA methylation, which in turn, may trigger the cascade of transcriptional silencing by targeting methyl-CpG-binding protein and histone deacetylases.

More strikingly, Zheng et al. report that HBx also plays a direct role in activation of the up-regulated genes. They convincingly show that DNMT3A (in the presence of HBx) dissociates from the promoter region of the up-regulated genes, thus resulting in observed hypomethylation and transcriptional activation. However, gene-specific differences are observed in the magnitude of induction of gene expression and also in levels of methyl-cytosine, suggesting that HBx, through its binding to DNMT3A (already associated with different gene-specific repression-complex subunits), may affect affinity of DNMT3A to the target regulatory DNA (Fig. 1C). Using ChIP experiments, authors failed to find recruitment of HBx on the activated target gene-promoters; hence it remains to be investigated, if HBx, could exhibit its transactivation effect through a mechanism other than that including recruitment of acetyltransferase activity (Fig. 1A). Given the vast possibilities of different cellular proteins exhibiting interaction with HBx, it calls for further investigations to unravel what factors govern these interactions.

While the epigenetic mechanisms involved in virus-associated cancers are poorly understood, recent advancements have linked a number of viruses (e.g. simian virus 40 T, Epstein–Barr virus and Kaposi's sarcoma-associated herpes virus), to the deregulation of DNMT family members through diverse mechanistic pathways, ultimately leading to methylation-based silencing or down-regulation of host cellular genes [6,7,9]. The work presented by Zheng et al. not only helps to gain a better understanding of molecular mechanisms of HBV: infection-related epigenetic aberrations, but also suggests that viral pathogens, in general, may promote carcinogenesis through similar epigenetic pathways.

Unlike genetic events, epigenetic events are reversible and hence hold better promise for therapeutic interventions. DNA-methyltransferase inhibitors and histone deacetylase inhibitors are already available as drugs to cure different cancers and are showing promising results. However, many of these epigenetic modulator drugs lack isoenzyme selectivity and thus are limited in their therapeutic value, due to their requiring high doses, resulting in toxic effects. Findings in the present study indicate that DNMT3A and HDAC1 specifically play a major role in epigenetic aberrations associated with HBV infection. Hence, it emphasizes the need for subclass-specific inhibitors of the epigenetic modulators, which may further help to narrow down the design of target-specific therapeutic strategies, thus reducing any side effects of epigenetic drugs. Moreover, these findings once again emphasize the role of the HBx-protein as a central player in HBV-induced epigenetic aberrations, contributing to the onset and progression of HCC. The large time gap between HBV infection and the onset of HCC, however, suggests that the virus-induced aberrant epigenetic profiles may not be a consequence of any targeted process. It is possible that epigenetic alterations induced by HBV set the stage for additional epigenetic as well as genetic events, which may be triggered by environmental and lifestyle factors.

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